

Prospective Validation for Prediction of Gefitinib Sensitivity by Epidermal Growth Factor Receptor Gene Mutation in Patients with Non-Small Cell Lung Cancer

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Introduction: We evaluated the efficacy of gefitinib monotherapy prospectively in patients with advanced or pretreated non-small cell lung cancer (NSCLC) harboring epidermal growth factor receptor (EGFR) mutations.

Methods: Patients with NSCLC were examined for EGFR exon 19 deletion mutations by fragment analysis and for EGFR L858R point mutations by the Cycleave polymerase chain reaction technique. EGFR mutation-positive patients with locally advanced, metastatic, or recurrent/refractory NSCLC that was not curable with surgery or thoracic radiotherapy were candidates for gefitinib treatment administered at 250 mg/day until disease progression.

Results: Mutations of the EGFR gene were detected in 27 (41%) of 66 patients. Ten had exon 19 deletion, and 17 had L858R. Twenty-one patients harboring EGFR mutations were treated with gefitinib and were considered assessable for responses and adverse events. Nineteen patients with EGFR mutations achieved objective responses (three complete responses and 16 partial responses), resulting in an overall response rate of 90.5% (95% confidence interval, 69.6%–98.8%). The median progression-free survival was 7.7 months (95% confidence interval, 6.0 mo to not reached). The median overall survival has not been reached. Common adverse events were skin toxicity, diarrhea, and elevated aminotransferases, but no pulmonary toxicity was observed.

Conclusions: Detection of common EGFR mutations seems to be useful for selecting patients with NSCLC who would likely benefit from gefitinib monotherapy.

Key Words: EGFR, Gefitinib, Lung cancer, Mutations, Drug sensitivity.

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Lung cancer remains the most common cause of cancer death in both men and women worldwide. Lung cancer frequently presents at an advanced and biologically aggressive stage, resulting in poor prognosis. Non-small cell lung cancer (NSCLC) accounts for more than 80% of all lung cancers. Currently, platinum-based combination chemotherapy regimens, including several active new chemotherapeutic agents, comprise the standard option for patients with advanced NSCLC. However, various combinations of drugs have similar efficacy, producing objective response rates of 30 to 40%, median survival time of eight to 10 months, and 1-year survival rates of 30 to 40%.^{1,2} These results remain unsatisfactory, and new modalities of treatment are urgently awaited. Recently, novel molecular targeted strategies that block cancer progression pathways have been suggested as the ideal treatment to control cancer and are considered an exciting therapeutic approach for treating NSCLC.³

The epidermal growth factor receptor (EGFR) is a 170 kDa receptor tyrosine kinase and a member of the erbB receptor family that plays a pivotal role in the signaling processes of tumor progression.^{4–6} EGFR is overexpressed in several solid tumors, including NSCLC, and it is one of the leading therapeutic molecular targets.⁷ Gefitinib is an orally bioavailable, selective EGFR tyrosine kinase inhibitor (TKI) and was the first targeted drug for NSCLC. Phase II and III monotherapy trials for patients pretreated for NSCLC demonstrated objective response rates of only 8 to 18%.^{8–10} However, subset analyses of these trials and a retrospective study¹¹ showed a small group of clinical responders comprising women, patients with adenocarcinomas, nonsmokers, and Japanese or Asian patients. These results suggest that identifying predictive molecular or genetic biomarkers for gefitinib sensitivity may be useful for selecting patients who are most likely to benefit from treatment.

In 2004, three independent groups reported that somatic EGFR mutations correlated with sensitivity of NSCLC to gefitinib or erlotinib, another EGFR TKI.^{12–14} Subsequently, several groups confirmed this striking correlation between EGFR mutations and gefitinib sensitivity, yielding a response rate of about 60 to 94% in retrospective analyses.^{15–22} EGFR mutations are likely to be significantly associated with survival benefit attributed to gefitinib treatment.^{17,18,21} In con-

trast to these results, recent reports concerning molecular analyses of large-scale phase II and III trials showed lower response rates than previously reported and no survival benefit in patients with mutations treated with TKIs.^{23–26} Around the same time, the EGFR gene amplification/copy number was demonstrated as another useful predictive molecular marker of TKI efficacy.^{23,26–28} However, these contradictory results were obtained through the retrospective collection of tumor samples, and prospective validation studies that predict TKI efficacy by EGFR mutations are needed.

Data from previous reports show that in-frame deletions in exon 19 and specific missense mutation of codon 858 in exon 21 (L858R) account for about 90% of all EGFR mutations, and about 80% of responders to gefitinib or erlotinib harbor either of these two hotspot mutations. Therefore, we developed a rapid, sensitive screening assay of two hotspot mutations²⁹ and conducted a prospective cohort study to explore the prediction of gefitinib sensitivity in EGFR mutation-positive patients.

MATERIALS AND METHODS

Study Design

This prospective cohort study was conducted to identify patients with NSCLC who would most likely benefit from gefitinib treatment according to their EGFR mutation. Patients with EGFR mutation were treated with oral administration of gefitinib at a dose of 250 mg once a day until disease progression or intolerable toxicity occurred, or until the patient refused to continue treatment. The primary endpoint was objective tumor response rate. Secondary endpoints included adverse effects, disease control rate (response + stable disease), progression-free survival (PFS), and overall survival (OS). This study was approved by the institutional review board of Aichi Cancer Center Hospital.

Patient Eligibility

Eligibility criteria for gefitinib treatment were adult (age ≥ 20 yr) with cytologic or histologic confirmation; locally advanced, metastatic, or recurrent/refractory NSCLC that was not curable by surgery or radiotherapy; harboring EGFR mutation; and one or more measurable or assessable lesions. All patients were admitted to the study regardless of prior treatment, extent of performance status (PS), or main organ functions. The exclusion criteria were pulmonary fibrosis, interstitial pneumonia, or prior treatment with an EGFR TKI or antibody. All patients gave written informed consent in accordance with institutional regulations before entering the study.

Efficacy and Toxicity Evaluation

Tumor responses were evaluated according to the Response Evaluation Criteria in Solid Tumors³⁰ and were confirmed by repeated imaging studies after 4 to 8 weeks of gefitinib treatment. During the treatment and for 30 days after the last dose of gefitinib, patients were monitored for adverse events, which were graded using Common Terminology Criteria for Adverse Events, version 3.0. PFS was assessed from the date of gefitinib treatment until the date of objective

disease progression, death from any cause, or the last follow-up. OS was assessed from the date of gefitinib treatment until the date of death from any cause, or the last follow-up.

Detection of EGFR Mutations

Genomic DNA was extracted from tumors embedded in paraffin blocks or from aspirated tumors obtained in pleural effusions, superficial lymph nodes, or subcutaneous metastasis. All specimens were reviewed by a single reference pathologist (Y.Y.) and marked grossly near the tumor-rich lesion on an unstained slide to enrich the tumor cell population as much as possible.

We performed mutational analyses of exon 19 deletion and the L858R point mutation of the EGFR gene, as previously described.²⁹ Briefly, exon 19 deletion was determined by common fragment analysis using polymerase chain reaction (PCR) with an FAM-labeled primer set, and the PCR products were electrophoresed on an ABI PRISM 310 (Applied Biosystems, Foster City, CA). The shorter segment of DNA amplified by PCR showed a deletion mutation in a new peak in an electropherogram. The L858R mutation was detected by the Cycleave real-time quantitative PCR technique using the Cycleave PCR core kit (Takara Co. Ltd., Ohtsu, Japan) with an L858R-specific cycling probe and a wild-type probe. Fluorescence intensity was measured with a Smart Cycler system (SC-100, Cepheid, Sunnyvale, CA).

Statistical Analysis

Data were analyzed using the chi-square test; $p < 0.05$ was regarded as significant. Confidence intervals (CI) were calculated using binomial CIs. PFS and OS were calculated using the Kaplan–Meier method and compared between two EGFR mutation groups using log-rank test. All the analyses were performed with Stata 8.2 for Macintosh (Stata Corp, College Station, TX).

RESULTS

Sampling Procedure for Detecting EGFR Mutations

Sixty-six consecutive patients with NSCLC were examined to detect the EGFR mutations from November 2004 through August 2005 at Aichi Cancer Center Hospital. Of these patients' samples, 23 specimens were obtained from bronchoscopic biopsy, 22 from computed tomography/ultrasound-guided needle biopsy, 13 from percutaneous aspiration (seven from pleural effusion, four from lymph nodes, and two from skin metastases), two from biopsy (one from tonsil metastasis and one from skin metastasis), and six from surgery with general anesthesia (three from thoracotomy, two from thoracoscopy, and one from mediastinoscopy (Table 1). Sixty samples (91%) were obtained from the biopsy or aspiration method. Tumor tissues or aspirates were procured at the time of initial diagnosis in 52 patients and at the time of tumor progression in 14 patients.

Patient Characteristics and EGFR Mutations

Mutations of the EGFR gene were detected in 27 (41%) of 66 patients. Ten of these had the deletion in exon 19, and

TABLE 1. Patient Characteristics and Sample Procurement According to EGFR Mutation Status

	EGFR Mutation Status			<i>p</i>
	All	Mutation	Wild type	
All cases	66	27 (21)	39	
Sex				0.175
Male	36	10 (8)	26	
Female	30	17 (13)	13	
Age (yr)				0.5084
≤64	31	14 (11)	17	
>64	35	13 (10)	22	
Histology				0.0199
Adenocarcinoma ^a	59	27 (21)	32	<i>p</i> (^a vs. ^b)
Squamous cell ^b	2	0	2	
Large cell ^b	2	0	2	
Pleomorphic ^b	1	0	1	
NSCLC NOS ^b	2	0	2	
Smoking status				0.0002
Never smoker ^c	24	17 (13)	7	<i>p</i> (^c vs. ^d)
Former smoker ^d	17	9 (7)	8	
Current smoker ^d	25	1 (1)	24	
Stage at initial diagnosis				0.6348
IA ^e	2	1	1	<i>p</i> (^e vs. ^f)
IIB ^e	4	2 (2)	2	
IIIA ^f	3	0	3	
IIIB ^f	16	3 (2)	13	
IV ^f	41	21 (17)	20	
Performance status				0.6059
0/1	51	20 (14)	31	<i>p</i> (0/1 vs. ≥2)
2	7	3 (3)	4	
3	3	1 (1)	2	
4	5	3 (3)	2	
Prior first treatment				ND
No	8	5 (5)	3	
Surgery	3	3 (1)	0	
Thoracic irradiation	4	2 (2)	2	
Chemoradiotherapy	10	2 (1)	8	
Bone irradiation	6	3 (3)	3	
Brain irradiation	6	3 (2)	3	
Sclerotherapy for effusion	1	1 (1)	0	
Chemotherapy	28	8 (6)	20	
Prior chemotherapy				0.4337
0	28	13 (12)	15	<i>p</i> (0 vs. ≥1)
One regimen	28	10 (6)	18	
Two regimens	8	4 (3)	4	
Three regimens	2	0	2	
Method for sample procurement				ND
Bronchoscopic biopsy	23	11	12	
CT/US-guided needle biopsy	22	6	16	
Pleural effusion aspiration	7	4	3	
LN/skin aspiration	6	2	4	
Tonsil/skin biopsy	2	0	2	
Thoracotomy	3	2	1	
VATS	2	1	1	
Mediastinoscopy	1	1	0	

EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; NOS, not otherwise specified; ND, not done; CT/US, computed tomography/ultrasound; LN, lymph node; VATS, video-assisted thoracoscopy. Superscript letters indicate groups compared in the statistical analysis. Numbers in parentheses represent the numbers of patients receiving gefitinib treatment.

17 were the point mutation at codon 858. As previously reported,^{12–14,17} the EGFR mutations were significantly associated with adenocarcinoma histology and never-smoking status (Table 1). However, the EGFR mutation status was not significantly correlated with sex, age, PS, stage at initial diagnosis, or prior chemotherapy. Twelve patients received gefitinib treatment as the first-line chemotherapy; five patients desired first-line gefitinib therapy, and the other seven were unfit for conventional chemotherapy because of age (one patient, age 84 yr), cardiac disease (one patient), widespread bone metastases (two patients), and poor PS (3–4 in three patients).

Clinical Response and Survival

Of 27 patients harboring EGFR mutation, 21 were treated with gefitinib and were assessable for objective responses (Table 2) and adverse events (Table 3). The median interval of gefitinib treatment was 5.9 months (range, 0.67 to 11.4 mo). Of the assessable 21 patients, 19 patients achieved objective responses (three complete response and 16 partial response), for an overall response rate of 90.5% (95% CI, 69.6–98.8%). One patient had stable disease, giving an overall disease control rate of 95.2% (95% CI, 76.2–99.9%). According to EGFR mutation classes and PS, the objective responses were seven of eight for the exon 19 deletion, 12 of 13 for the L858R point mutation, 13 of 14 in PS 0 to PS 1 patients, and 6 of seven in PS 2 to PS 4 patients. The response to gefitinib did not differ significantly according to the mutation class or PS.

The median PFS was 7.7 months (95% CI, 6.0 mo to not reached) (Figure 1A). The median OS has not been reached at present (Figure 1B). Subset analyses showed that PFS was greater in patients with the exon 19 deletion than in those with the L858R point mutation (log rank test, $p = 0.04$; Fig 2A). The median PFS for the exon 19 deletion group was 7.8 months (95% CI, 7.6 mo to not reached); for the L858R mutation group, median PFS was 6.0 months (95% CI, 2.6 to 7.7 mo). OS did not differ significantly between the two types of mutations (Figure 2B). No difference was observed in PFS

TABLE 2. Response of EGFR Mutation-Positive Patients to Gefitinib Treatment

	EGFR Mutation Status		
	Exon 19 Deletion (<i>n</i> = 8)	L858R Mutation (<i>n</i> = 13)	Total (<i>n</i> = 21)
CR	1 (12.5%)	2 (15.4%)	3 (14.3%)
PR	6 (75%)	10 (76.9%)	16 (76.2%)
Overall response rate (CR + PR)	7 (87.5%)	12 (92.3%)	19 (90.5%)
SD	1 (12.5%)	0	1 (4.8%)
Disease control (CR + PR + SD)	8 (100%)	12 (92.3%)	20 (95.2%)
Progressive disease	0	1 (7.7%)	1 (4.8%)

EGFR, epidermal growth factor receptor; CR, complete response; PR, partial response; SD, stable disease.

TABLE 3. Number (%) of Patients with Treatment-Related Adverse Events (*n* = 21)

	Grade				
	0	1	2	3	4
Skin toxicity	15 (71)	4 (19)	2 (10)	0	0
Diarrhea	13 (62)	3 (14)	3 (14)	2 (10)	0
Elevated aspartate aminotransferase/ alanine aminotransferase	15 (71)	1 (5)	2 (10)	3 (14)	0
Nail changes	17 (81)	3 (14)	1 (5)	0	0
Mucositis	20 (95)	1 (5)	0	0	0
Joint pain	20 (95)	1 (5)	0	0	0

and OS between never-smokers and current/former smokers (data not shown).

Adverse Events

All 21 patients were evaluated for drug-related adverse events. The most common adverse events were skin toxicity, diarrhea, and elevated aspartate aminotransferase/alanine aminotransferase (AST/ALT) (Table 3). The grade 3 adverse events of diarrhea and elevated AST/ALT occurred in two (10%) and three (14%) patients, respectively. These events occurred slightly more frequently than in previous studies.^{8,9} No grade 4 adverse events or pulmonary toxicity were observed. Seven patients required an interruption of treatment, lasting 2 to 4 weeks, because of grade 2/3 diarrhea or grade 3 elevated transaminases. Two patients withdrew: one after 3 weeks of gefitinib treatment because of grade 3 diarrhea, and the other after 9 weeks of gefitinib treatment because of grade 2 nail changes.

DISCUSSION

In the present study, we have observed that the objective response rate in our patients was similar to that in previous reports. We also found that PFS and OS seem promising in identifying gefitinib-sensitive patients regardless of whether the study includes patients unsuited for conventional cytotoxic chemotherapy because of age, cardiac disease, widespread bone metastases, or poor PS (3 to 4). Our favorable data might have resulted because we selected patients harboring one of two hotspot mutations (exon 19 deletion and exon 21 L858R mutation). Greulich et al.³¹ examined NIH-3T3 cells transformed with various EGFR mutants and showed that a distinct EGFR mutation confers differential sensitivity to TKIs. They demonstrated greater sensitivity to TKIs in cell lines with the two hotspot mutations than with the G719S mutation, and insensitivity to TKIs in cell lines with exon 20 insertion (D770-N771 ins) mutation. These in vitro data may explain, at least partially, our promising results for detecting these two sensitive mutations.

We previously reported that patients with the EGFR exon 19 deletion respond significantly better to gefitinib than those with the L858R mutation ($p = 0.0108$).¹⁷ Our current data show no difference in gefitinib sensitivity and OS after

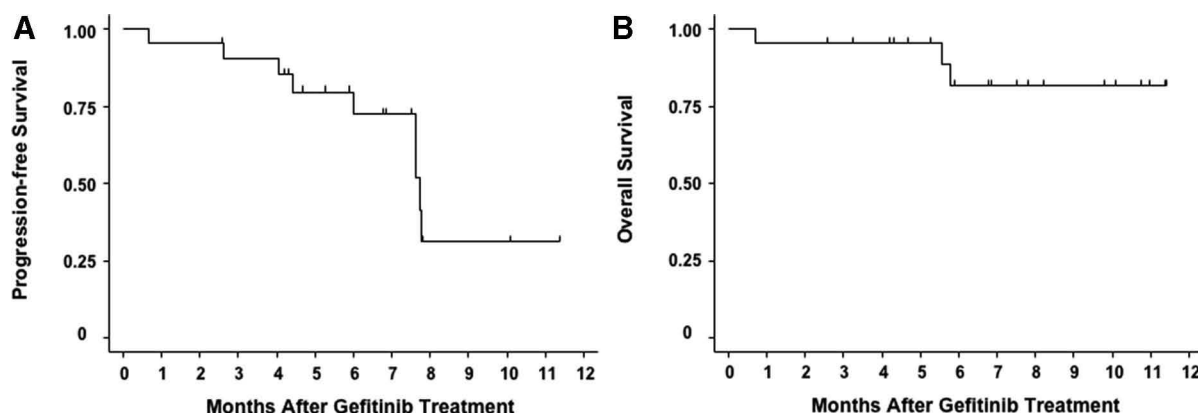


FIGURE 1. Kaplan-Meier estimates of (A) progression-free survival and (B) overall survival for patients with EGFR mutations ($n = 21$). The median progression-free survival was 7.7 months (95% CI, 6.0 mo to not reached). The median survival was not reached.

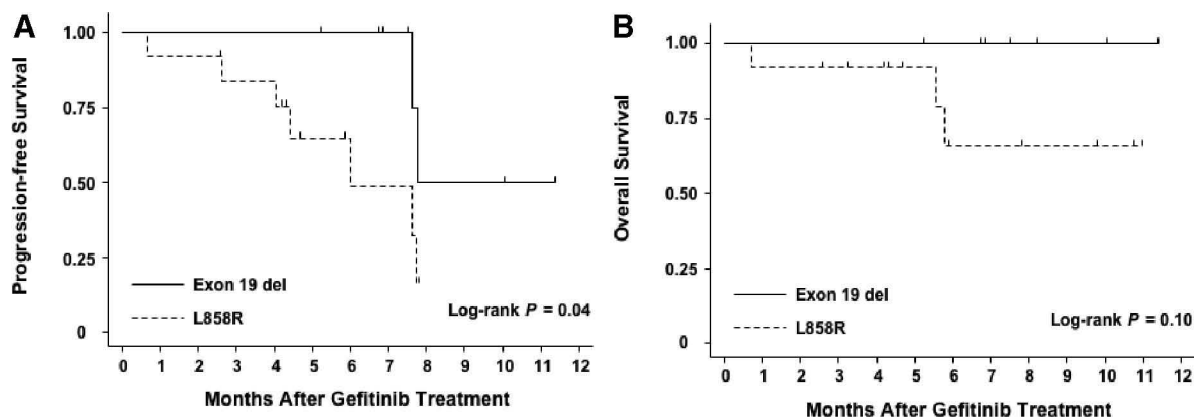


FIGURE 2. Kaplan-Meier estimates of (A) progression-free survival and (B) overall survival for patients with EGFR mutations according to the exon 19 deletion ($n = 8$) and L858R mutation ($n = 13$). The median PFS for the exon 19 deletion group was 7.8 months (95% CI, 7.6 mo to not reached); for the L858R mutation group, median PFS was 6.0 months (95% CI, 2.6 to 7.7 mo).

gefitinib treatment between these two groups of patients, although we observed a greater PFS in the EGFR exon 19 deletion group than in the L858R group. It is possible that the number of patients (eight with exon 19 deletion and 13 with L858R) was too small to detect a statistically significant difference in OS. Riely et al.³² reported recently that patients with exon 19 deletion have a significantly longer survival after TKI treatment than those with the L858R mutation ($p = 0.01$). These findings suggest that the EGFR exon 19 deletion might be a better predictor of the efficacy of TKIs than the L858R mutation.

EGFR mutations are significantly associated with patients with adenocarcinomas, patients of Asian origin, females, and patients who had never smoked—clinical factors also associated with patients who respond to gefitinib.^{13,14,24,33} A phase II trial using gefitinib monotherapy as the first-line therapy for patients with adenocarcinoma histology and never-smoking status was recently completed in South Korea and reported promising data (e.g., an objective response rate of 69% and estimated 1-year survival rate of 73%).³⁴ However, this trial did not select patients using

biomarkers, and we believe the benefit of gefitinib therapy could be enhanced by selecting individual patients according to appropriate biomarkers. Very recently, two prospective phase II studies that had selected patients based on molecular biomarkers demonstrated that EGFR mutations³⁵ and gene copy number assessed by fluorescence in situ hybridization (FISH)³⁶ can predict clinical outcomes in TKI-treated NSCLC patients.

The grade 3 adverse events of diarrhea and elevated AST/ALT were observed in five patients (24%); this is a higher rate than that reported in two previous phase II studies that reported rates of adverse events of 1.5%⁸ and 7%⁹ at a gefitinib dose of 250 mg per day. The reasons for our higher rate of adverse events are unknown. Although adverse events related to gefitinib treatment are generally thought to be mild and tolerable, they should not be discounted.

Most studies have detected EGFR mutations using direct sequencing or single-strand conformation polymorphism analysis for exons 18 to 21.³⁷ These techniques are less sensitive when applied to a small amount of tumor cells from the biopsy or aspiration samples.³⁸ We were able to detect

two hotspot mutations with our sensitive rapid screening assay in most biopsy or aspiration samples in the routine clinical setting. Although this assay needs precise assessment of tumor samples by a pathologist to enrich the tumor cells, it is very sensitive and accurate for detection, and it can be completed within 4 hours without need for microdissection or nested PCR process.²⁹

The key genetic event for TKI sensitivity has not been perfectly identified and is the subject of a growing debate about the role of EGFR mutations versus EGFR gene amplification/copy number in NSCLC. EGFR mutant NSCLC cell lines are strongly associated with increased EGFR gene copy number.^{39,40} Cappuzzo et al.²⁷ and Takano et al.²² found that EGFR mutations in NSCLC patients correlate significantly with gene copy number assessed by FISH and quantitative real-time PCR, respectively. However, Cappuzzo et al.²⁷ demonstrated that in patients treated with gefitinib, a high EGFR gene copy number is a better predictor of survival than EGFR mutations.²⁷ In contrast, Takano et al.²² reported that the status of the EGFR mutations, rather than gene copy number, is the major determinant of gefitinib efficacy. Recent reports of the molecular analyses from the largest phase III TKI monotherapy trials failed to show that the EGFR mutation is superior to gene copy number in predicting the efficacy of TKIs.^{23,26} These conflicting results on EGFR mutations and gene amplification/copy number could be explained by (i) differences in the detection methodologies and assessment of mutation and gene amplification/copy number (e.g., direct sequence versus PCR-based DNA testing for detecting EGFR mutations, or FISH versus PCR-based amplification for detecting EGFR gene amplification/copy number), (ii) failure to reconfirm these results in other institutions, and (iii) other unknown factors underlying drug sensitivity, especially those related to ethnicity. Further prospective studies are needed to investigate the crucial molecular markers involved in the EGFR network, using adequate tissue samples and assays to more precisely detect molecular events.

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